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Toxicokinetics of T-2 Toxin, HT-2 Toxin and T-2 Triol after Intravenously Administrated T-2 Toxin in Swine

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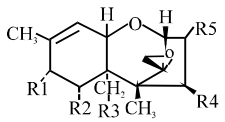
Abstract: Toxicokinetics of trichothecene mycotoxin, T-2 toxin, HT-2 toxin and T-2 triol were determined in seven swine, after intravenous administration (i.v.) of T-2 toxin (0.5 mg kg^{-1}). The T-2 toxin, HT-2 toxin and T-2 triol plasma concentrations were analyzed by HLPC-MS/MS from 2.5 min to 6 h. The mean pharmacokinetic parameters for T-2 toxin, HT-2 toxin and T-2 triol were respectively: half-life ($t_{1/2\lambda Z}$) 11.7, 37.7 and 25.6 min; areas under the plasma concentration-time curves (AUC_{0-00}): 16499, 4619 and 11011 min ng mL^{-1}. Following i.v administration, times to observed maximal concentration (Tmax) were 2.5, 2.5 and 7.5 min, respectively. No T-2 tetraol was detected at all sampling times. The total body Clearance (Cl) and the apparent steady-state volumes of distribution (V_{ss}) for T-2 toxin were 0.662 L kg^{-1} and 0.038 L/min/kg. This study demonstrated that T-2 toxin is rapidly removed from the blood followed by its fast conversion to metabolites.

Key words: Toxicokinetics, T-2 toxin, metabolites, swine, blood, parameters

INTRODUCTION

T-2 toxin [4α-diacetoxy-8β-(3-methylbutyryloxy)-12,13-epoxy-trichothec-9-en-3α-ol] is the most potent toxic mycotoxin of the group trichothecenes type A produced by fungi of the *Fusarium* genus, i.e., *Fusarium acuminatum*, *Fusarium poae* and *Fusarium sporotrichioldes* (Buck and Cote, 1991) which naturally occur in various raw agricultural produce (wheat, maize, barley, oats and peanuts) [corn = maize] (Desjardins *et al*., 1993). Along with T-2 toxin_Ctypical metabolites of T-2 toxin in body fluids and organism, HT-2 toxin (hydrolysis), T-2-triol (hydrolysis), T-2-tetraol (hydrolysis) (Fig. 1) are also of importance, those substances could be detected in food and feed samples and are also formed *in vivo* after consumption (Yagen and Bialer, 1993; Schollenberger *et al*., 2006). Due to its acute and chronic toxic effects including vomiting, diarrhoea, skin irritation, neuro-endocrine changes, bone marrow aplasia and immune modulation (Raisbek *et al*., 1991), T-2 toxin is a potential health risk in human nutrition (WHO, 1990). T-2 toxin is considered a major agent in causing many diseases such as Alimentary Toxic Aleukia (ATA) of man (Mirocha and Pathre, 1973) mouldy corn toxicosis of cattle (Hsu *et al*., 1972), fusariotoxicosis of fowl (Greenway and Puls, 1976) and bean-hulls poisoning of horses (Ueno *et al*., 1972). It also has been associated with outbreaks of mycotoxicosis in human and farm



Metabolites	R1	R2	R3	R4	R5
T-2 toxin	$OC(O)OCH_2CH(CH_3)_2$	H	$OCOCH_3$	$OCOCH_3$	OH
HT-2 toxin	$OC(O)OCH_2CH(CH_3)_2$	H	$OCOCH_3$	OH	OH
T-2 triol	$OC(O)OCH_2CH(CH_3)_2$	H	OH	OH	OH
T-2 tetraol	OH	H	OH	OH	OH

Fig. 1: Structure of T-2 toxin and some its typical metabolites

animals in the United States (Hsu *et al*., 1972), Canada (Puls and Geenway, 1976), Japan (Ueno, 1977), the Soviet Union (Joffe and Yagen, 1978) India (Bhat *et al*., 1989) and China (Wang *et al*., 1993).

Due to human consumption of T-2-contaminated food, especially foods of animal origin, studies on distribution and metabolism of T-2 toxin and its dominant metabolites have been of great concern. Kinetic studies on T-2 toxin and its metabolisms *in vivo* have been reported in swine (Beasley *et al*., 1985; Corley *et al*., 1985, 1986; Bernhoft *et al*., 2000), chickens (Yoshizawa *et al*., 1980; Giroir, 1990), cows (Chatterjee *et al*., 1986; Yoshizawa *et al*., 1981; Visconti *et al*., 1985) and dogs (Sintov *et al*., 1986, 1988). According to these studies the half-life of T-2 toxin in plasma is <20 min for there occurs rapid deacetylation to HT-2 toxin and in small

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amount to T-2-triol. T-2 and its metabolites generally accumulate weakly in animal tissues. The toxicokinetic results were different for different species, administration routes and doses.

Although, much information on the metabolism fate of T-2 has been obtained in various animals through radio-labeled T-2 administration, very little is known about pharmacokinetics of T-2 toxin in swine. In this study researchers hope to determine the pharmacokinetic features of T-2 toxin and its metabolites HT-2, T-2 triol and T-2 tetraol with the application of a new analytical method.

MATERIALS AND METHODS

Chemicals: T-2 toxin (T-2) and HT-2 toxin (HT-2) standard power, T-2 triol (triol) and T-2 tetraol (Tetraol) stock standard solutions (50 μg mL^{-1}, 1 mL) and ammonium acetate (MS grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Acetonitrile and methanol (HPLC grade) were purchased from Merck (Germany) while all the other chemicals were of analytical grade and made in China. Ultrapure water was produced by a Millipore Milli-Q System (Millipore, Bedford, MA, USA).

Stock standard solutions (T-2 and HT-2) were prepared by dissolving each substance in acetonitrile (50 μg mL^{-1}). The T-2 solution for i.v. (5 mg mL^{-1}) was prepared by dissolving the T-2 standard in 50% ethanol-water (5:5v/v).

Animals and experimental design: Seven clinically-healthy growing pigs (Duroc-Landrace-Yorkshire) weighing 6.8±0.5 kg ($\bar{X} \pm SE$) were bought from one aquaculture plant in Guangdong province. The piglets were housed in semi-contained pens with access to water *ad libitum* and commercial non-medicated feed at scheduled times. Each pig received a single intravenous injection of 0.5 mg kg^{-1} T-2 via the auricular vein. Blood samples, each 3 mL were collected from the *vena cava* prior to the i.v. inoculation and at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 120, 240 and 360 min thereafter. All samples were immediately frozen and stored at -20°C until analyzed.

LC-MS/MS analysis: LC-MS/MS analysis was performed on a Sciex API 4000 System (Applied Biosystems, USA) with a 1100 Series LC System (Agilent Technologies, Waldbronn, Germany). Separation was performed on ZORBAX Eclipse XDB-C18 columns (150×2.1 mm, 3.5 μm) (Agilent Technologies, USA).

Mobile Phase A consisted of acetonitrile/water, 5/95 (v/v), containing 5 mM ammonium acetate while mobile Phase B consisted of acetonitrile/water, 60/40 (v/v) also containing 5 mM ammonium acetate. Elution with mobile Phase A and a linear gradient was applied, reaching 100% mobile Phase B after 1 min (holding time: 5.5 min) and then switched back (6.6 min) to mobile Phase A (holding time: 5.4 min) which was maintained till the end of the run at 12.0 min. A volume of 5 μL was injected into the chromatographic system. The column flow rate was 250 μL min^{-1} and the column temperature was kept at 30°C.

The ESI interface used positive ion modes at 650°C with the following settings: CUR 20 psi, GS1 55 psi, GS2 50 L min^{-1}, ionization voltage +5000V, CAD 5 psi, DP 54V, EP 10V, CE 24V, CXP 12V. The dwell time was 200 ms.

Sample preparation: Plasma sample (0.5 mL) was transferred to a 4 mL centrifuge tube, a volume of 0.5 mL acetonitrile for protein precipitation was added to the tube and then was vortexed for 1 min . After centrifugation for 12 min at 12000 rpm, the supernatant was filtered through a nylon centrifuge filter (0.22 μm). Final samples were stable for at least 5 days when stored at 4-8°C.

Data analysis: For quantification, the peak-area was measured and the external standard method used. Data acquisition and processing were performed using Analyst Software 1.5 (Applied Biosystems). The pharmacokinetic analysis of T-2, HT-2 and triol was performed using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA, USA). A no-compartment approach of IV-BOLUS was chosen to analyze the plasma toxin concentration-time data. The λ_Z is a first-order rate constant associated with the terminal segment of the curve. It was estimated by the linear regression of the terminal data points. The terminal elimination half-life ($t_{1/2\lambda Z}$) was calculated by $t_{1/2\lambda Z} = 0.693/\lambda_Z$. Areas under the plasma concentration-time curves (AUC_{0-00}) were calculated by the method of trapezoids. The total body Clearance (Cl) was calculated from $Cl = Dose_{i.v.}/AUCi.v$ and the apparent steady-state volume of distribution (V_{ss}) was calculated using $V_{ss} = (Dose_{i.v.})\ (AUMC)/AUC^2$, Mean Residence Time (MRT) was calculated from MRT = AUMC/AUC. All measurements are given as mean values±SE.

RESULTS AND DISCUSSION

The ranges of linearity for assaying T-2, HT-2 and triol were: 0.5~500, 1~200 and 10~500 ng mL^{-1} which always yielded a correlation coefficient exceeding 0.998. The within-run and inter-run precisions for T-2, HT-2 and

triol were 2.54~4.78, 3.23~5.12 and 3.46~5.79%, respectively. Limits of quantification (S/N = 10/1) were 0.5, 1 and 10 ng mL^{-1}, respectively.

Clinically, the body of pigs initially showed trembling, especially in the hind limbs and five pigs developed diarrhea, persistent vomiting, chewing and odontoprisis at about 12 min after intravenous administration ed of T-2. The clinical symptoms continued for about 30 min. The animals appeared to adapt to the toxin and became clinically normal again within the experimental period.

Figure 2 shows the fate of T-2, HT-2 and triol in swine plasma after intravenously administrating T-2 (0.5 mg kg^{-1}). T-2, HT-2 and triol decreased rapidly in the plasma of the intravenously dosed swine and the disposition was biphasic. The plasma T-2 and triol levels dropped quickly within 20 min following administration and then declined to lower level. However, HT-2 was eliminated relatively slower.

Toxicokinetic parameters (mean values of individual parameters) determined for the piglets dosed i.v. are shown in Table 1. The maximum plasma concentrations (C_{max}) f T-2 and triol were present at first sampling time (2.5 min) after dosing, namely, 1588±298 and 771.1±182 ng mL^{-1}, respectively. The HT-2 concentration attained C_{max} (76.51±11.9 ng mL^{-1}) at 7.5 min. T-2 and HT-2 could not be detected or the concentration was lower than LOD at 4 h following i.v. administration, triol even was at 1.5 h. T-2 and HT-2 concentrations (Fig. 2a and b) were 2.697±0.286 and 11.56±0.48 ng mL^{-1}. No tetraol was detected at all sampling times and the 5 min sample from number 3 pig was lost. Compared with its metabolites, the T-2 parent concentration was higher at initial sampling times. However, T-2 was eliminated fast, the order of elimination half-lives ($t_{1/2\lambda z}$) being HT-2 (37.7) >triol (25.6) >T-2 (11.7 min). The Cl and V_{ss} of T-2 was were 0.038±0.0098 l/min/kg and 0.66±0.19 L kg^{-1} (Table 1), respectively.

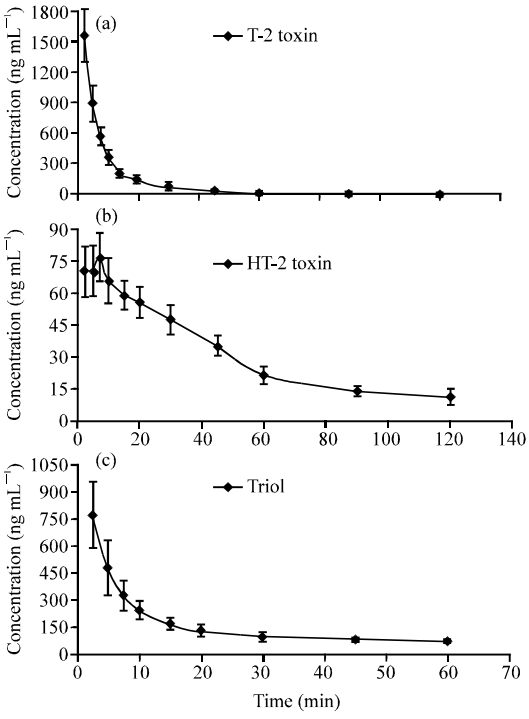

Fig. 2: a) Plasma concentration-time curve for T-2 toxin in swine following i.v. T-2 toxin administration; b) Plasma concentration-time curve for HT-2 toxin in swine following i.v. T-2 toxin administration; c) Plasma concentration-time curve for triol in swine following i.v. T-2 toxin administration. The dose was 0.5 mg kg^{-1}. Results are shown as Mean±SE (n = 7)

In this study, we determined the toxicokinetics of T-2 toxin, HT-2 toxin and triol after a single intravenous administration 0.5 mg kg^{-1} b.w. pure T-2 in seven experimental pigs, this species being the most important in providing animal origin food in China. The main highlight of the research is that researchers obtained the time-course of T-2 and its typical metabolites HT-2 and triol simultaneously. To the best knowledge, no such studies have ever been reported. As shown in Fig. 2, the pharmacokinetic profiles of T-2 and triol were similar but a little different from HT-2 and the plasma T-2 and triol levels were much higher than that of HT-2.

Therefore, HT-2 as one intermediate metabolite was very unstable *in vivo*. Less than 2% of the total plasma amount was found as HT-2 toxin in the monkey at all time points (Naseem *et al*., 1995). It has been reported that $t_{1/2\lambda Z}$ of T-2 was 13.8 min in swine (Beasley *et al*., 1985) which is in accordance with the result of the present study (11.7 min) and demonstrates that T-2 was deacetylated reapidly to HT-2 and other metabolites. Sintov *et al*. (1988) reported the half-lives of HT-2 in whole blood and plasma *in vitro* to be 0.84 and 7 h, respectively. Maybe

Table 1: Pharmacokinetic parameters of T-2 toxin, HT-2 toxin and T-2 triol in swine following a single intravenous injection of pure T-2 toxin (0.5 mg kg^{-1})

Parameters	Unit	T-2	HT-2	T-2 triol
λ_Z	L min^{-1}	0.061±0.0049	0.0192±0.0013	0.0320±0.043
$t_{1/2\lambda Z}$	min	11.70±0.79	37.7±2.44	25.6±2.9
AUC_{0-00}	min×ng mL^{-1}	16499±2356	4619±574	11011±1394
MRT	min	10.82±1.72	46.68±7.4	29.8±4.1
C_{max}	ng mL^{-1}	1558±298.4	76.51±11.43	771.1±182.4
T_{max}	min	2.5	7.5	2.5
V_{ss}	L kg^{-1}	0.662±0.192	-	-
Cl	L/min/kg	0.038±0.0098	-	-

$t_{1/2\lambda Z}$: The elimination half life; AUC_{0-00}: Area Under the Curve; MRT: Mean Residence Time; C_{max}: Maximum concentration; T_{max}: Time to reach observed maximum concentration; Cl: The total body clearance; V_{ss}: The apparent steady-state volume of distribution

some enzymes present in blood cells play an important role in the degradation of these toxins. The $t_{1/2\lambda Z}$ of T-2 in dog was 5.8 min after i.v. administration (0.4 mg kg^{-1}) while that of HT-2 was 19.6 min (Sintov *et al*., 1986). So, the fate of T-2 vary greatly among different species.

CONCLUSION

No tetraol was detected in plasma, the reason might be that very small amount was produced and part of it presented as glucuronic acid conjugates. In this study, researchers also did signal oral administration in two pigs at the same dosage but no parent compound and only the typical three metabolites were detected which demonstrated a very active first-pass effect and/or intragastric microbes (what microbes can survive in pig stomach? It would be safer to use enzymes) degraded these toxins. Further research is needed to elucidate the distribution and elimination of these toxins in pig tissues. From the findings we would like to determine which of the major metabolites could be used as clinical indicators of T-2 intoxication.

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